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Original Article

Assessing the Subchronic Toxicity of the Ethanolic Extract of the Pods of *Tetrapleura tetraptera* on Swiss Albino Mice

Oloyede Adeola Michael[1*]

[1] Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba, Lagos State, Nigeria

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Abstract

Background and Aim: *Tetrapleura tetraptera* fruit is widely used in south-east and western Nigeria not only as a spice but also as a remedy for various ailments. This research aims at examining the subchronic toxicity of the ethanolic extract of the pods of *T. tetraptera* on Swiss albino mice.

Materials and Methods: Twenty-five Swiss albino Wistar mice weighing between 18-30g were randomly divided into five groups. Animals in group 1 that was regarded as the control group were administered distilled water. Animals in group 2-4 were orally administered 50,100 and 200mg kg^{-1} bodyweight of *T. tetraptera.* All animals were fasting on the eve of the 43rd day. Following the withdrawal of treatment, the blood, liver, kidneys, lungs and heart were removed for hematologic, biochemical and histologic investigations.

Results: No remarkable alteration was observed in the hematologic parameters except for the significant reduction in the WBC. The biochemical investigation indicated gross changes in most of the biochemical analytes dose-dependently. The levels of ALT, ALP, TB, DB, PRO, CREA, UREA, ALB and CHOL were all noticeably ($P<0.05$) different from the control group in all doses which might be indicative of toxicity. Moreover, some minor changes were observed in the histoarchitecture of the liver and the kidney.

Conclusion: Hence, the results revealed that the extract might have induced toxicity to the kidneys and liver which may in turn influence other parts of the body. *Tetrapleura tetraptera* may contain some phytochemicals, which on prolonged use may affect the physiology of the body.

Keywords: *Tetrapleura tetraptera*, Subchronic toxicity, Hematology, Histology, Biochemical analytes

***Corresponding Author:** Oloyede Adeola Michael, Department of Cell Biology and Genetics, University of Lagos, Akoka-Yaba, Lagos State, Nigeria. Email: oloism@yahoo.com.

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Introduction

Tetrapleura tetraptera (Schum & Thonn), Taubert (*Fabeceae*) is a tree that grows in the tropical jungles of West Africa in which the trees lose their leaves annually. It is abundantly found in Nigeria (1). *T. tetraptera* is locally referred to by various names by different ethnic groups in Nigeria. It is referred to as Aidan in English, Osakirisa or Oshosho in Igbo, Aridan in Yoruba, Dawo in Hausa, Abogolo in Igala, and Osirisa in Ikwo dialect (1). In the Eastern parts of Nigeria, *T. tetraptera* fruits are used to prepare soups for mothers from the first day of delivery to prevent postpartum contraction. It is also an important

component of pot-herbs, and is widely used in the preparation of pepper soup in Southern Nigeria (2) .Many researchers have investigated the medicinal significance of the plant for the treatment of various human illnesses (3). Studies have reported the anti-microbial activities of *T. tetraptera*, and also demonstrated the presences of oxalates, cyanogenic glycosides, tannins and phytic acids in the plant (4). It has also the capability of healing wounds (5). *T. tetraptera* has been shown to affect some hematologic profiles (6). The fruit infusion is consumed as a restorative tonic and memory enhancer in the traditional African medicine. Hence, it has anticataleptic properties, and also enhances antioxidant defense systems (7). An investigation of the pod indicated that it contained high amounts of polyphenol, flavonoid saponin, tannin and phytate (8). Another research revealed the presence of crude protein, sugar and starch. According to the dry weight, the mineral content (mg/kg) was Fe, Zn, Cu, Mg, Mn, Na, Ca, K and B (8). The subchronic toxicity of the pods of *T. tetraptera* has not been adequately examined so far. This study, therefore, was designed to expose the impact of the oral administration of crude ethanolic extract of the pods in Swiss albino mice.

Materials and Methods

Plant Material

T. tetraptera pods were purchased from Bariga market, Lagos state, Nigeria and were identified and authenticated by the Forestry research institute, Ibadan Voucher No .FHI10798.

Preparation of *T. tetraptera* Extract

The pods of *T. tetraptera* were air dried under shade for 96 hours and ground to powder by an electric grinder. The powder was, then, weighed with an electronic weighing balance that indicated the weight to be 1000g. It was soaked in 2.5 liters of absolute ethanol and covered with aluminum foil for 48 hours in a glass jar at room temperature. After 48 hours, the mixture was filtered with muslin cloth to remove all debris. The filtered mixture was poured into three beakers and then placed on a hot stirrer plate at 40±1°C. A paste-like form of the mixture was obtained and then freeze drying was performed. This was scraped from the beakers with a spatula and weighed on a weighing balance. 130.4g of crude ethanol extract was obtained.

Experimental Animals and Diet

Twenty-five albino Swiss Wistar male mice, weighing between 18-30 kg were purchased from the Nigerian Institute of Medical Research (NIMR), and were maintained at the animal house of the botanical garden of the University of Lagos, Cell Biology and Genetics Department. The animals were kept in a cycle of 12 hours of light and 12 hours of darkness, in 22±1°C temperature and 55±5% relative humidity. The mice were kept in well-ventilated cages with iron mesh covers and wood shaving floors. They were fed daily with rat pellets and allowed access to water ad libitum. The mice were acclimatized for two weeks. They were randomly divided into five equal groups, i. e, five mice per group. The body weight of each group was recorded before the commencement of treatment and on weekly basis. After the withdrawal of the treatment, the animals fasted overnight, and were subsequently sacrificed under light ether anesthesia on the next day. The study conforms to the requirements of 21 CFR 58: US FDA Good Laboratory Practices (GLP) Standards, 1987, and OECD Principles of GLP, 1997 (9)

Collection of Blood and Organs

On the 43rd day, all animals were sacrificed under anesthesia with light ether in a desiccator. This 43-day research aimed at producing beneficial information to be used in the design for spermatogenic and chronic toxicity studies. This is to ensure to the extent possible that proper doses are utilized and animals not underexposed. The blood samples of the mice were collected via jugular puncture into labeled bottles of Ethylenediaminetetraacetic acid (EDTA) and Heparin for haematology and biochemical analysis. The collected blood was allowed to coagulate via remaining at room temperature and being centrifuged. The serum (Supernatant) was isolated and stored until it was analyzed. All animals were subjected to autopsy, during which all organs except the testes, stomach and intestines were collected and preserved in universal bottles containing Bouin's solutions which were labeled accordingly.

The biochemical and hematological analysis were carried out using standard assay kits from Randox Chemicals, UK, using Bayern instruments.

Histology Preparation

Histological tissue studies of the accessory organs from each animal were fixed in Bouin's solution and prepared for hematoxylin-Eosin staining. Photomicrographs of the prepared slides hematoxylin-Eosin stained tissue sections were taken with a camera attached to the compound light microscope.

Statistical Analysis

The results were analyzed using Student's t-test.

Ethics statement

Ethical approval was received from ethical committee of the University of Lagos, College of Medicine, University of Lagos in November 2016.

Results and Discussion

Clinical Observation

Throughout the experiment, the mice did not show any observable sign of toxicity or morbidity as they looked healthy. They were fed well and their faces looked normal.

There is no significant difference in the weight of the internal organs of the mice from the control group.

There are significant differences in the biochemical analysis of the treated mice compared to the control group.

The biochemical investigation of the liver (Table 3) showed that most of the analyses were significantly different from the control group in dose-dependent fashion. ALT, ALP, TB, DB, PRO, CREA, UREA, ALB and CHOL were all significantly $P<0.05$ different from the control group in all doses which might indicate toxicity.

T. tetraptera did not show a remarkable difference in hematological analysis between treatment and control (Table 4). Hb and PCV at 200mg kg^{-1} were significantly increased, whereas WBC decreased at 200 and 100mg kg^{-1}, though insignificant, and at the same time reduced significantly at 50mg kg^{-1}

Histological Results

In this sub-acute toxicity study in mice, the ethanolic extract of *T. tetraptera* did not induce any change in animal behavior, food and water intake, and vital organs weight as well as the body weight gain. It is widely acknowledged that reduction of the body weight and internal organ weights is a sensitive index of toxicity following the exposure to toxic materials (10). Moreover, increase or decrease in body weight could be due to the adverse effects of drugs (10). The loss of appetite caused by stress or physiological adaptation to a drug's intake could lead to the reduction of caloric intake when the body weight reduces (11) or body fat accumulation during body weight gain (12). The hematopoietic system is highly susceptible to toxic chemicals and is also a significant index of physiological and pathological conditions in humans and animals (13). Most of the hematological parameters that were studied did not show significant difference from the control group. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) indicated insignificant decreases in the extract treated compared to the control group. Hence, the results of MCV that were obtained from hematological studies imply the possible loss of fluid in treated mice since increased albumin, as observed in biochemical investigation and reduced MCV values, are markers of dehydration (14).

Being capable of decreasing MCV, especially at the highest dose group, *T. tetraptera* makes the plant material efficient in the treatment of macrocytic anaemia (15). However, it could also make normocytic patients microcytic, which is a dangerous blood disorder. Nonetheless, it has been reported that MCH levels mirror the MCV level; hence, justifying the remarkable reduction observed in the level of MCH in the highest dose group (16). RBC exhibited a significant increase in the highest dose indicating that the extract may not induce anaemia. The significance of calculated blood indices in anaemia diagnosis has been reported (17). Furthermore, there was no significant rise in WBC count which is known to increase as body defense in response to toxic environments (18). Lymphocyte, the main effector's cell of the immune system (19) recorded fluctuation indicating that the extract might not have brought about any challenge on the immune system of the animals (18). However, Hb and PCV were significantly different from the control group. Hematologic investigation provides a significant indication of local and systemic intoxication manifestations caused by drugs (20).

Table 1: Body Weights of the Control Group and Treated Groups.

Weeks	Control	50mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}
1	18.90 ± 0.81	22.26 ± 0.88	23.66 ± 0.71	21.36 ± 1.04
2	21.22 ± 1.00	20.62 ± 0.50	23.08 ± 1.29	21.34 ± 1.02
3	21.92 ± 1.38	21.32 ± 0.99	24.02 ± 1.63	21.68 ± 0.62
4	22.48 ± 2.17	20.60 ± 4.05	21.50 ± 1.29	20.90 ± 1.45
5	20.96 ± 1.95	26.17 ± 4.43	21.78 ± 1.46	22.45 ± 1.73
6	20.73 ± 1.89	24.60 ± 3.07	22.33 ± 1.33	25.48 ± 1.47

Data are expressed as Mean ± SEM. $p<0.05$ significantly different from control.

Table 2: The Effects of the Oral Administration of *T. tetraptera* on the Relative Internal Organ Weight.

Treatment	Heart(g)	Spleen(g)	Lungs(g)	Kidney(g)	Liver(g)
Control	0.08±0.01	0.21±2.73	0.14±0.01	0.15±0.01	0.86±0.07
50 mg kg-1	0.06±0.01	0.09±0.01	0.11±0.03	0.13±0.01	0.71±0.10
100 mg kg-1	0.08±0.00	0.22±0.04	0.10±0.01	0.18±0.01	0.89±0.06
200mg kg-1	0.08±0.00	0.11±0.02	0.12±0.10	0.17±0.01	0.70±0.02

Values are mean ± SEM (N=5/group),*$p<0.05$ significantly different from control.

Table 3: The Biochemical Analysis of the Liver and Blood Sample of Mice Administered with *T. tetraptera.*

Treatment	AST	ALT	ALP	TB	DB	PRO	CREA	UREA	ALB	CHOL	TRIG	HDL	LDL
Control	37.92±0.72	35.13±0.31	8.37±0.15	0.60±0.03	0.10±0.01	37.93±0.64	0.28±0.03	21.69±0.92	4.43±0.05	88.16±1.54	55.82±2.75	52.03±1.19	24.97±2.38
50 mg kg^{-1}	42.24±2.76	47.82±0.36^{c}	12.97±1.18^{a}	0.82±0.03^{b}	0.18±0.01^{a}	43.67±0.66^{b}	0.35±0.02^{a}	27.76±1.34^{a}	2.84±0.03^{c}	81.49±0.85^{a}	55.05±1.32	55.24±0.51^{a}	15.24±0.58^{a}
100 mg kg^{-1}	70.88±13.52	41.48±2.98	14.51±0.75^{a}	1.06±0.05^{a}	0.21±0.01^{a}	51.63±0.10^{b}	0.44±0.02^{c}	34.90±0.81^{b}	3.45±0.04^{a}	89.64±0.85^{b}	87.94±2.02^{a}	25.33±0.77^{a}	46.41±1.27^{b}
200 mg kg^{-1}	98.77±1.25^{c}	47.94±0.41^{b}	14.16±0.49^{c}	0.93±0.03^{a}	0.18±0.01^{c}	49.66±0.22^{b}	0.32±0.04^{c}	37.41±0.49^{a}	2.61±0.02^{c}	112.36±1.30^{c}	96.35±2.65^{a}	29.70±0.44^{b}	63.39±2.15^{c}

Values are mean ± SEM (N=5/group), $^{a}p< 0.05$; $^{b}p<0.005$; $^{c}p<0.0001$ significantly differed from the control group.

Table 4: The Hematological Analysis of Mice Administered with *T. tetraptera.*

Treatment	Hb	PCV	RBC	WBC	MCV	MCH	MCHC	Neut	Lymph	Mono	Eos	Bas
Control	10.93±0.53	29.33±1.76	3.53±0.19	6866±881.9	82.89±1.01	30.26±0.28	36.50±0.37	30±0.57	66.33±0.88	2.0±0.57	1.66±0.33	0
50 mg kg^{-1}	10.93±0.68	30±2.08	3.61±0.34	3516±303.2^{a}	83.48±2.64	30.46±1.11^{a}	36.47±0.24	26.3±2.18	72±2.64^{a}	1.0±0.57	0.66±0.66	0
100 mg kg^{-1}	10.9±0.49	30±1.52	3.39±0.10	6333±148.1	85.72±0.81	31.16±0.14^{a}	36.35±0.23	16±1.73^{a}	80.66±0.88^{b}	2.0±0.57	1.33±0.33	0
200 mg kg^{-1}	13.94±0.16^{a}	39±0.57^{a}	4.93±0.20^{a}	5950±427.2	79.18±2.21	28.31±0.83^{a}	35.75±0.10	27±1.15	70.33±0.88	2.33±0.33	0.33±0.33^{a}	0

Values are mean ± SEM (N=5/group) $^{a}p< 0.05$; $^{b}p<0.005$; $^{c}p<0.0001$ significantly differed from the control group.

The liver and kidneys are two essential organs that have significant roles in detoxification (21). It has been reported that some herbal mixtures have hepatotoxic and nephrotoxic effects (8). As the integrity and functionality of the liver and the kidneys as homeostatic organs are lost, clinical chemistry parameters, including serum enzymes and analytes, increase.

The effect of ethanolic extract of *T. tetraptera* was investigated on biochemical parameters such as ALT, AST, ALP, which are essential for assessing the hepatic function (10), and on urea and creatinine levels in the blood which are usually used to evaluate the kidney function (22). A rise of transaminases may indicate liver inflammation or damage (23), whereas any rise in creatinine level suggests damages of nephrons function (10). The subchronic oral administration of the crude ethanolic extract of *T. tetraptera* in mice brought about significant alterations of the biochemical profile when compared to the

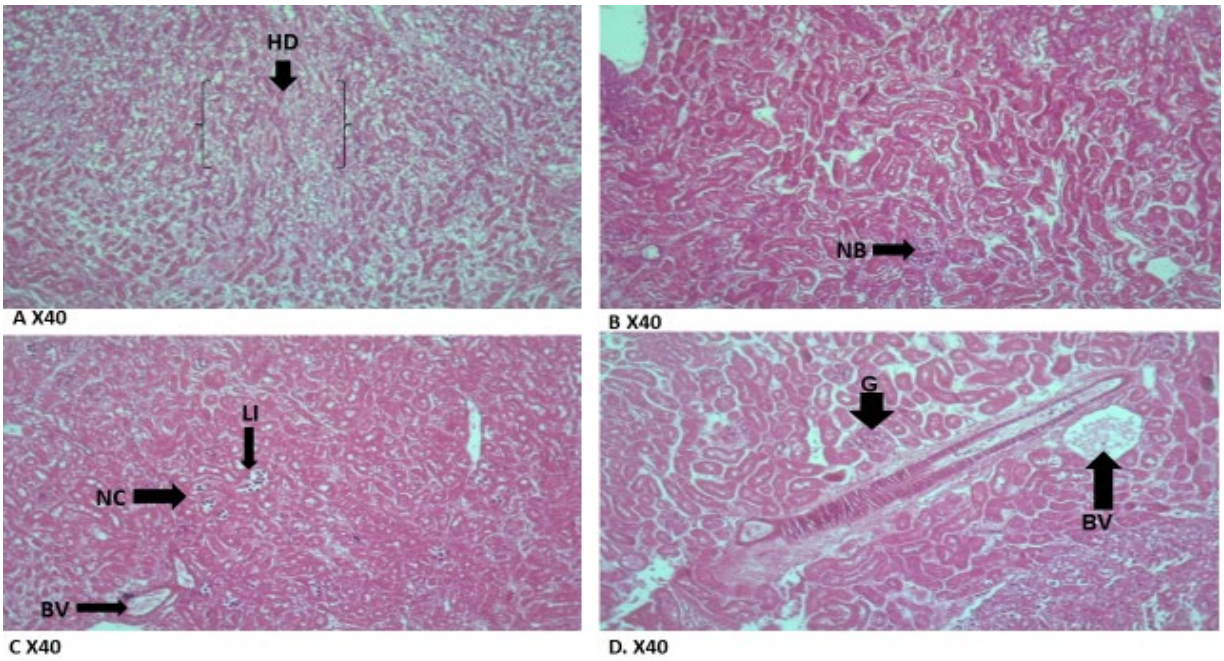

Figure 1. Histological samples of the kidney of mice administered with varied doses of *T. tetraptera* extract revealed dose-dependent alterations. a) 50mg/kg^{-1}, showing HD- hyalination and tubular degeneration. b) 100mg/ml, showing NB-narrow bowman space. c) 200mg/kg^{-1}, showing NC- narrow bowman capsule and glomerular congestion, LI- leucocyte infiltration. BV- blood vessel d) Control, G- glomerulus, BV- blood vessel. All tissues are conserved.

control group. The increase in ALT and AST serum blood levels and liver homogenates in comparison to the control group may indicate injury to hepatocytes, nephrocytes and other organs. ALT is a more specific marker of damage to liver cells, for it takes place more often in the liver while AST is also found in heart, skeletal muscle, kidneys, brain, pancreas and blood cells (24). Hence, the rise in serum ALP might be regarded as a sign of cholestasis, which may result from intracellular hepatic canaliculi obstruction associated with inflammation (3). In the liver, ALT is limited to cytoplasm, whereas AST is found in both mitochondria (80% of total intracellular enzyme) and cytoplasm (20%) (25). In patients suffering from kidney disease, the AST and ALT are considerably lower than in healthy individuals because of reduced free pyridoxal-5-phosphate, which decreases the enzymatic activity (26).

As a sensitive organ, the kidney has a significant function which is affected by a number of factors, including drugs such as phytochemicals of plant origin that finally results in renal failure (22). The rise of creatinine and urea level implies that the plant extract affects the renal function. With regard to other biochemical parameters, albumin which increased significantly, is also an important protein synthesized in the liver and serves as a significant biomarker for liver ailments (16). Albumin maintains osmotic pressure, protein reservoir and transportation of endogenous and exogenous substances (27).

In toxicological studies

Histopathological investigations provide convincing evidences for biochemical and hematological observations (8). Histopathological investigation, therefore, indicated minor changes of the kidney and liver organs from control animals. Remarkable changes in the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, creatinine, TB, DB, protein, albumin and cholesterol which are appropriate indicators of liver and kidney roles, imply that the sub-chronic administration of the extract changed hepatocytes and nephrocytes of mice and may have also affected the normal metabolism of the animals. The fact that these clinical blood chemical parameters are the indexes of kidney and liver functions suggests that the extract may have induced toxicity to the kidneys and liver. These observations were further confirmed by the slight alteration in the histoarchitecture assessment of the organs.

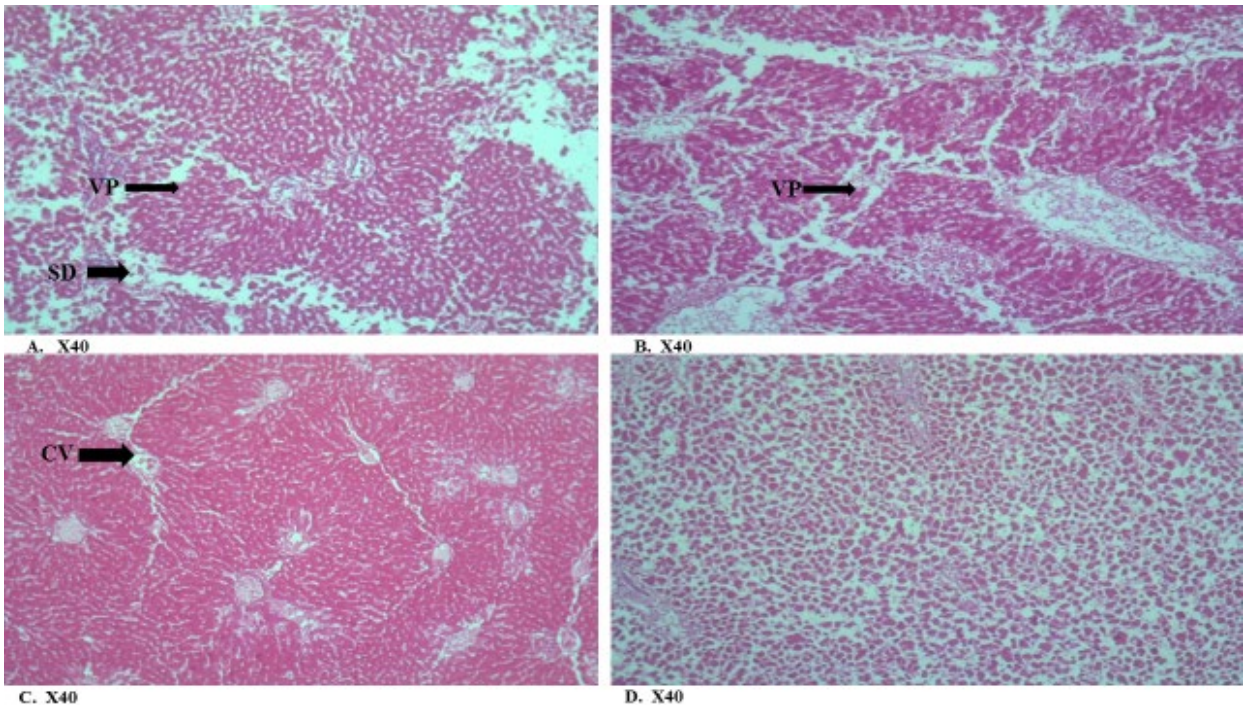

Figure 2. Histological sections of the liver of mice administered with varied doses of *T. tetraptera* extract. a) 50mg/kg^{-1}, showing VP-vacuolar perivascular degeneration and SD- sinusoid dilation b) 100mg/kg^{-1}, VP-Vacuolar perivascular degeneration. c) 200mg/kg^{-1}, congestion of CV- central vein and sinusoids. d) Control, tissues are conserved.

Conclusion

This study indicated that *T. tetraptera*, at the doses administered and for the period of administration, could induce minor toxicity on the internal organs and the biochemical analytes. Hence, it can be suggested that the presence of phytochemicals may be deleterious when administered for a prolonged period. However, further studies should be carried on in vitro to ascertain the veracity of toxicity on tissue culture.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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